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14. ABSTRACT

Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate. However, the luminal origin of prostate cancer has been challenged by a number of recent publications. This project was proposed to determine whether prostate cancer cells are derived from luminal or basal epithelial cells using genetic lineage tracing of prostate carcinogenesis in PSA-CreER^{T2};R26RmT/mG;EAF2-/-;PTEN+/- mice. Unfortunately, the PSA-CreER^{T2};R26RmT/mG;EAF2-/-;PTEN+/- mice died at young age unexpectedly and prevented us from performing the proposed experiments. As an alternative, we have generated PSA-CreERT2;R26RmT/mG;EAF2-/- mice to determine the origin of mPIN, the putative precursor of prostate cancer. These animals will be sacrificed and analyzed during 2016.

15. SUBJECT TERMS Nothing listed

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Introduction

Defining the origin of prostate cancer cells is fundamentally important and will guide future research to focus on cells from which prostate cancer cells are derived. Prostate cancer is thought to be derived from luminal epithelial cells in the prostate, because a hallmark of prostate cancer is the loss of basal epithelial cells and prostate cancer cells exhibit a luminal epithelial cell phenotype including the expression of AR and PSA [1]. The capability of luminal epithelial cells as origin of prostate cancer is also supported by over expression of oncogenes such as cMYC and T-antigen or knockout of important tumor suppressors such as PTEN, specifically in prostate luminal epithelial cells. However, the luminal origin of prostate cancer has been challenged by a number of recent publications [2, 3]. This project will determine whether prostate cancer cells are derived from luminal or basal epithelial cells in an EAF2-/- mouse model, and determine whether luminal-derived prostate cancer cells behave differently from basal-derived prostate cancer cells.

Body

Genetic lineage tracing [4, 5] [6] and the EAF2-/- mouse prostate cancer model [7] will be used to determine whether prostate cancer cells are derived from basal and/or luminal epithelial cells in the prostate *in vivo*. All mice in this study will be on a C57BL/6J background.

Original Task 1: To determine whether prostate cancer can be derived from luminal epithelial cells in the EAF2-/-;PTEN+/- mouse prostate cancer model using the PSA-CreER^{T2}-based genetic lineage tracing (months 1-30)

A. Obtain IACUC approval and generate PSA-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/- mice (month 1-16)

We have written and received approval for IACUC protocol 12020202. We have initially generated 7 breeding pairs (consisting of 1 male and 1 female) with the following genotypes: PSA-CreER^{T2}; EAF2-/-;PTEN+/- mated to R26RmT/mG. These breeding pairs were established to generate 80 male PSA-CreER^{T2}; R26RmT/mG;EAF2-/-;PTEN+/- mice required to complete Specific Aim 1. The first litters of pups from these mice consisted of 28 pups of which 1 male and 1 female had the following phenotype: PSA-CreER^{T2}; R26RmT/mG;EAF2+/-;PTEN+/- (Figure 1). These mice will be utilized as a breeding pair to enhance the efficiency of breeding. Additionally, a cohort of 10 male R26RmT/mG mice generated through the breeding strategy were being utilized to determine the rate of spontaneous labeling in the prostate following multiple cycles of regression and regrowth. A subgroup of 4 animals were castrated at 10 weeks of age and will be subjected to 4 rounds of regression and regrowth. These animals were examined to determine what percentage, if any, of epithelial cells can be labeled through spontaneous recombination. These animals will serve as additional experimental controls.

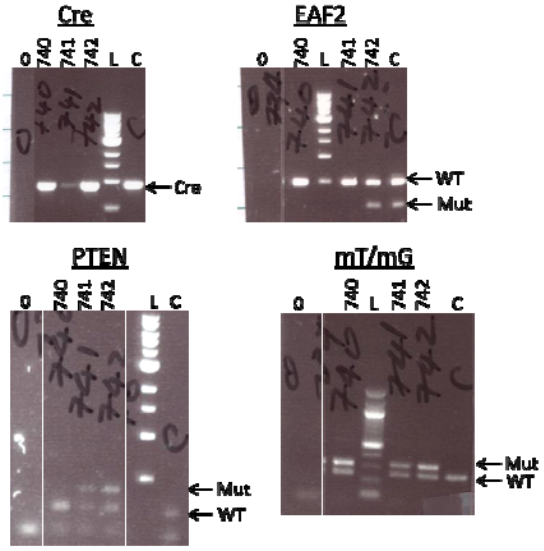

Figure 1. Genotypic analysis of offspring from PSA-CreER^{T2}; EAF2-/-;PTEN+/- mated to R26RmT/mG mice. Tail DNA was isolated from offspring at weaning and examined by PCR. Animal #742 is PSA-CreER^{T2}; R26RmT/mG;EAF2+/-;PTEN+/-. Molecular weight ladders are shown (L) as well as negative (0) and positive controls (C).

In the funding year 2013, we generated breeding pairs consisting of: (a) 1 female PSA-CreER^{T2}; EAF2-/-;PTEN+/-;R26RmT/mG+/- and 1 male EAF2-/-;PTEN+/-; R26RmT/mG+/-; (b) 1 female EAF2-/-;PTEN+/-; R26RmT/mG+/- and 1 male PSA-CreER^{T2}; EAF2-/-;PTEN+/-;R26RmT/mG+/-. The breeding pairs were identified in Table 1. Increased death has been noted in several animals with the following genotypes: PSA-CreER^{T2}; EAF2-/-;PTEN+/- at 1-2 mos of age, and PSA-CreER^{T2}; EAF2+/-;PTEN+/- at 6-12 mos of age. These mice were deceased due to unknown reasons. This has prevented the generation of the final cohort of male PSA-CreER^{T2}; R26RmT/mG;EAF2-/-;PTEN+/- mice required to complete Specific Aim 1.

Table 1. Specific Aim 1 breeding pairs 2014

ID	Sex	DOB	PSA-CreERT2	EAF2	PTEN	R26RmT/mG
25	F	1/8/14	+	-/-	+/-	+/-
746	M	10/14/13	-	+/-	+/-	+/-
20	F	11/26/13	-	-/-	+/-	+/-
1	M	10/14/13	+	-/-	+/-	+/-

A subgroup of 4 male R26RmT/mG mice generated through the breeding strategy was utilized to determine the rate of spontaneous labeling in the prostate following multiple cycles of regression and regrowth. This subgroup of 4 animals was castrated at 10 weeks of age and subjected to 4 rounds of regression and regrowth as additional experimental controls. In the absence of the PSA-CreER^{T2} transgene, no labeled epithelial cells were detected in any of the R26RmT/mG animals (Figure 2). These results are in agreement with previous description of the R26RmT/mG murine model [8].

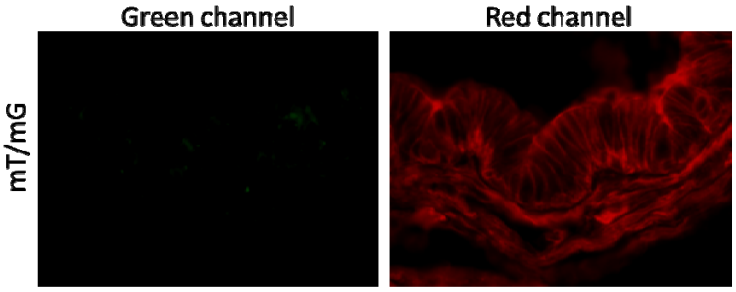

Figure 2. Images of fixed tissue sections of mT/mG male mice after 4 cycles of regression and testosterone-stimulated regrowth demonstrating minimal background fluorescence of mT and mG into the opposite channels.

B. Genotyping to verify genotype of genetically modified animals (month 1-18).

Genotyping of pups was performed, tail clippings were taken for genotyping analyses at 21 days of age at weaning. See Figure 1.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 4-20)

D. Histological and genetic lineage analysis of prostate cancer in mice (month 15-30)

Revised Task 1. To determine whether prostatic intraepithelial neoplasia can be derived from luminal epithelial cells in the EAF2-/- mouse PIN model using the PSA-CreER^{T2}-based genetic lineage tracing. (months 1-48)

A. Modified breeding strategy to generate a total of 15 male PSA-CreER^{T2};R26RmT/mG;EAF2-/- mice. (months 26-36)

To address the unanticipated early death of PSA-CreER^{T2};R26RmT/mG;EAF2-/-;PTEN+/- animals, which was originally proposed, the breeding strategy and sample groups was streamlined and modified. The modification was approved by DoD. All 15 animals have been generated and will be sacrificed at 12 mos of age, from 10/15/15 through 6/12/16.

E. Group	Mouse ID	DoB	Tamoxifen	Date at Sac. (12 mos)	Purpose
1	505	3/12/15	+	3/12/16	Origin of PIN
	507	3/12/15		3/12/16	
	576	5/21/15		5/21/16	
	583	5/24/15		5/24/16	
	589	6/12/15		6/12/16	
	590	6/12/15		6/12/16	
	594	6/12/15		6/12/16	
	595	6/12/15		6/12/16	
	67	10/15/14		10/15/15	
	56	10/15/14		10/15/15	
2	530	4/15/15	-	4/15/16	Control
	541	4/23/15		4/23/16	
	546	4/23/15		4/23/16	
	562	5/8/15		5/8/16	
	68	10/15/14		10/15/15	

Table 2. Number of PSA-CreER^{T2};R26RmT/mG;EAF2-/- male mice in 2 groups. Sac., Sacrifice

B. Genotyping to verify genotype of genetically modified animals. (months 26-36)

All genotyping is complete.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells when mice are 6 weeks of age. (months 30-38)

All tamoxifen injections have been completed.

D. BrdU injection 4 hours prior to sacrifice. (months 40-46)
The experiments are ongoing as the animals age.

E. Histological and genetic lineage analysis of prostate cancer in mice. (months 36-48)
The studies will be initiated after all animal tissues are collected.

<u>Original Task 2: To determine whether prostate cancer cells can be derived from basal epithelial cells in the EAF2-/-;PTEN+/- mouse prostate cancer model (months 10-32).</u>

A. Generate CK5-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/- mice (month 10-20)

We encountered difficulties with importing the CK5- or CK14-CreER^{T2}. The CK5-CreER^{T2} mice at Dr. Brigid Hogan's lab in Duke were positive with virus, and our animal facility was unable to accept the mice. Dr. Xin Li from Baylor has kindly offered us CK14-CreER^{T2} mice. Unfortunately, these animals were also infected with virus. Luckily, CK5-CreER^{T2} became available at Jackson Lab. We placed order immediately and it took about 6 months for them to revive the colony and ship the animals to us. Crosses of between CK5-CreER^{T2} and EAF2-/- mice were initiated and the first generation of CK5-CreER^{T2}; EAF2+/- consisted of 6 offspring (Figure 3). Our research progress was significantly delayed due to the difficulties in the importing the CK5-CreER^{T2} mice. Also, we had to use PSA-CreER^{T2}; EAF2-/-; R26RmT/mG instead of

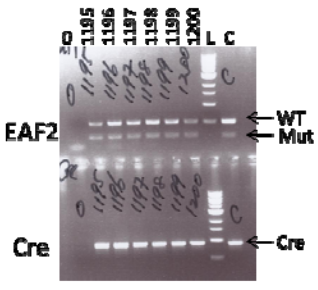

Figure 3. Genotypic analysis of offspring from CK5-CreER^{T2} mated to EAF2-/- mice . Tail DNA was isolated from offspring at weaning and examined by PCR. Animals are CK5-CreER^{T2}; EAF2+/-. Molecular weight ladders are shown (L) as well as negative (0) and positive controls (C).

PSA-CreER^{T2}; EAF2-/-;PTEN+/-;R26RmT/mG in the lineage tracing of luminal epithelial cells. Thus, we decided to use CK5-CreER^{T2}; R26RmT/mG; EAF2-/- instead of CK5-CreER^{T2}; R26RmT/mG; EAF2-/-; PTEN+/- in the lineage tracing of basal epithelial cells.

B. Genotype genetically modified animals (month 12-22).

Genotyping of pups was completed, tail clippings were taken for genotyping analyses at 21 days of age at weaning.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark luminal epithelial cells (month 14-20).

D. Histological and genetic lineage analysis of prostate cancer in mice (month 16-32)

Revised Task 2. To determine whether prostatic intraepithelial neoplasia can be derived from basal epithelial cells in the EAF2-/- mouse PIN model using the CK5-CreER^{T2}-based genetic lineage tracing. (months 10-48)

A. Modified breeding strategy to generate a total of 15 male CK5-CreER^{T2};R26RmT/mG;EAF2-/- mice (revised as for Task 1A). (months 26-36)

The breeding strategy and sample groups were streamlined and modified to address the unanticipated early death of genetically modified mice with PTEN+/-. In the revised Task 2, we proposed to generate 15 CK5-CreER^{T2};R26RmT/mG;EAF2-/- mice, which was approved by DoD. Currently, 10 (out of 10) animals in Group 1 and 5 (out of 5) in Group 2 have been generated and will be sacrificed at 12 mos of age, from 3/12/16 through 9/16/16.

Group	Mouse ID	DoB	Tamoxifen	Date at Sac. (12 mos)	Purpose
1	394	3/18/15	+	3/18/16	Origin of PIN
	406	3/18/15		3/18/16	
	448	5/26/15		5/26/16	
	907	7/8/15		7/8/16	
	917	7/16/15		7/16/16	
	918	7/16/15		7/16/16	
	922	7/16/15		7/16/16	
	935	8/8/15		8/8/16	
	947	8/24/15		8/24/16	
	959	9/4/15		9/4/16	
2	373	12/26/14	-	12/26/15	Control
	375	1/2/15		1/2/16	
	428	5/1/15		5/1/16	
	937	8/11/15		8/11/16	
	971	9/16/15		9/16/16	

Table 3. Number of CK5-CreER^{T2};R26RmT/mG;EAF2-/- male mice in 2 groups. Sac., Sacrifice

B. Genotyping to verify genotype of genetically modified animals. (months 12-36)
Genotyping verification was completed.

C. Intraperitoneal injection of tamoxifen to induce genetic combination to mark basal epithelial cells when mice are 6 weeks of age. (months 30-38)
Intraperitoneal injection of tamoxifen was completed.

D. BrdU injection 4 hours prior to sacrifice. (months 40-46)
The experiments are ongoing as the animals age.

E. Histological and genetic lineage analysis of prostate cancer in mice. (months 36-48)
The studies will be initiated after all animal tissues are collected.

Original Task 3: To determine whether prostate cancer cells derived from luminal epithelial cells are different from those from basal cells in the EAF2-/-;PTEN+/- mouse prostate cancer model (months 16-36).

A. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in luminal-derived prostate cancer (month 20-32).

B. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers, and neuroendocrine markers in basal-derived prostate cancer (month 20-32).

C. Determine the effect of castration on luminal-derived and basal-derived prostate cancer (month 16-36).

D. Data analysis and manuscript writing (month 30-36).

Revised Task 3: To determine whether prostatic intraepithelial neoplasia derived from luminal epithelial cells are different from those derived from basal cells in the EAF2-/- mouse PIN model.
(months 36-54)

A. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers and neuroendocrine markers in luminal-derived PIN lesions. (months 36-48)

B. Immunohistochemical analysis of cell proliferation, luminal markers, basal markers and neuroendocrine markers in basal-derived PIN lesions. (months 36-48)

C. Determine the effect of castration on luminal-derived and basal-derived PIN lesions. (months 30-38)

D. Data analysis and manuscript writing. (months 48-54)

The Task 3 will be started once all the prostatic tissues are collected.

Key Research Accomplishments
- Obtained approval of the IACUC protocol for this project.
- Generated 6 breeding pairs of PSA-CreER^{T2}; R26RmT/mG;EAF2+/-;PTEN+/-. Unfortunately, these mice died at young age due to unknown reasons, which prevented us from performing the originally proposed tasks and caused a significant setback.
- Generated 15 animals for PSA-CreER^{T2}; EAF2-/-; R26RmT/mG+/- (See Table 2).
- Obtained CK5-CreER^{T2} mice.
- Generated 15 CK5-CreER^{T2}; EAF2-/-; R26RmT/mG+/- male mice (See Table 3).
- Determined that mG labeling does not spontaneously occur in mT/mG animals subjected to multiple cycles of prostate regression and testosterone-stimulated regrowth (See Figure 2).

Reportable Outcomes
None.

Conclusions
PSA-CreER^{T2};R26RmT/mG;EAF2-/-;PTEN+/- mice died at early age unexpectedly, which prevented us from performing experiments as originally proposed. We have modified the experimental design, which was approved by the PCRP program, to use PSA-CreER^{T2};R26RmT/mG;EAF2-/- and CK5-CreER^{T2};R26RmT/mG;EAF2-/- mice. We have generated all of the animals proposed in the revised Tasks. The experiments are expected to be completed in 2016.

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